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The cytotoxic effect of pinostrobin fingerroot (*boesenbergia pandurata*) on the culture of hela cells

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ABSTRACT

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Background: Cancer is one of the leading causes of death worldwide, therefore struggles to find more effective treatment and prevention is needed. Several studies has been performed using natural ingredients, one of which is Temu Kunci (*B. pandurata*). Temu Kunci extract contains flavonoid pinostrobin that has been showed as having cytotoxicity effects. Cytotoxicity tests of pinostrobin have been performed on several tumor cell lines, but its cytotoxicity effect on HeLa cell line has never been reported.

Objective: To assess cytotoxicity effect of pinostrobin temu kunci on HeLa cell culture

Methods: This study used simple experimental design. Pinostrobin were isolated from temu kunci and proved by TLC densitometry compared to standard pinostrobin. HeLa cell culture were treated with pinostrobin with concentrations 5, 25, 50, 75, 100, and 250 ug/mL. Cytotoxicity test were performed by MTT assay. Data were analyzed using one-way ANOVA.

Results: There was significant difference ($p=0.000$) of means of cell viability percentage, respectively: 92.58 ± 9.84 (5 μ g/mL), 91.78 ± 4.4 (25 μ g/mL), 80.09 ± 4.51 (50 μ g/mL), 76.89 ± 7.75 (75 μ g/mL), 67.85 ± 11.31 (100 μ g/mL), dan 48.82 ± 16.61 (250 μ g/mL). The IC₅₀ was 250 μ g/mL.

Conclusion: Pinostrobin showed no active cytotoxicity effect on HeLa cell culture.

Latar Belakang: Penyakit kanker merupakan salah satu penyebab morbiditas dan mortalitas yang tinggi hampir di seluruh dunia, sehingga diperlukan berbagai upaya untuk pencarian pengobatan dan pencegahan yang lebih efektif. Beberapa penelitian dilakukan dengan menggunakan bahan yang berasal dari alam. Salah satu bahan alam Indonesia yang banyak diteliti adalah tumbuhan rimpang Temu Kunci (*B. pandurata*). Ekstrak tumbuhan tersebut mengandung antioksidan flavonoid pinostrobin yang diketahui memiliki daya toksisitas terhadap sel kanker, tetapi belum diketahui bagaimana daya sitotoksitasnya terhadap kultur sel HeLa.

Tujuan: Untuk mengkaji daya sitotoksik pinostrobin temu kunci terhadap kultur sel HeLa.

Metode: Penelitian ini menggunakan desain eksperimental murni. Pinostrobin diisolasi dari rimpang temukunci dan dibuktikan dengan analisis densitometri TLC dibandingkan dengan

pinostrobin standar. Kultur sel HeLa diberi penambahan pinostrobin dengan konsentrasi 5, 25, 50, 75, 100, dan 250 µg/ml. Sitotoksitas dinilai berdasar hasil MTT assay. Data yang didapat dianalisis menggunakan one-way ANOVA.

Hasil: Didapatkan hasil perbedaan yang signifikan ($p=0,000$) dari rerata persentase viabilitas sel HeLa masing-masing; $92,58 \pm 9,84$ (5µg/mL), $91,78 \pm 4,4$ (25µg/mL), $80,09 \pm 4,51$ (50µg/mL), $76,89 \pm 7,75$ (75µg/mL), $67,85 \pm 11,31$ (100µg/mL), dan $48,82 \pm 16,61$ (250µg/mL). Didapatkan IC50 sebesar 250µg/mL.

Kesimpulan: Pinostrobin tidak menunjukkan daya sitotoksitas pada kultur sel HeLa.

INTRODUCTION

Temu kunci or fingerroot (*Boesenbergia pandurata* Roxb.) is one of Indonesian traditional herbal medicine plant which has a lot of health benefits, such as for mucolytic antitussive medicine, antacids, appetite enhancer, anti-stomatitis, and galactagogue. A lot of research has been done to determine the health potentials of fingerroot rhizome and essential oil, and it has been proven to have anti-fungal^{1,2} anti-bacterial^{3,4}, antiviral^{5,6}, anti-inflammatory⁷, and antioxidant properties^{8,9,10}. Previous researches that have been done on colon and breast cancer cells showed a promising potential of fingerroot as anti-tumor.¹¹ This finding is in line with a study by Kamkaen et al., who found that fingerroot extract had cytotoxic properties against laryngeal cancer cells (Hep2).¹²

The active ingredient contained in fingerroot that is thought to have anti-tumor potentials is flavonoid pinostrobin (5-hidroksi-7-metoksiflavanon) which bioactivities had been widely studied as anti-oxidant¹³, antiviral¹⁴, and anti-tumor. The anti-tumor effect of pinostrobin had been examined by Le Bail et al.¹⁵ on breast cancer cells. Smolarz et al. studied the effect of pinostrobin on the apoptotic respond of in vitro leukemia cells.¹⁶ The effect of pinostrobin on HeLa cells is not yet known.

METHODS

Study design and subjects

This study is a laboratory experimental study to compare the anti-cancer potentials of pinostrobin fingerroot based on its cytotoxicity on HeLa cell cultures. HeLa cell cultures were obtained from LPPT Hayati Universitas Gadjah Mada Yogyakarta. Pinostrobin used in this study was isolated from fingerroot rhizome (*B. pandurata*) by Laboratorium Biologi Farmasi FMIPA Universitas Islam Indonesia. The determination of fingerroot plant was also done by Laboratorium Biologi Farmasi FMIPA UII Yogyakarta

Study Procedure

a. Fingerroot preparation and pinostrobin isolation

Temukunci or fingerroot (*B. pandurata*) used in this study was obtained and processed into powder by Merapi Farma company. The identification of the plant and isolation of pinostrobin was done at Fakultas MIPA Universitas Islam Indonesia.

The dry powder of fingerroot rhizome (400g) was extracted 3 times using n-hexane. The dregs from the extraction process were re-extracted using ethyl acetate 3 times. Then, the extracts were evaporated using rotary evaporator until a thicker extract was gained (20,3g). The outcome of these processes undergo Vacuum Liquid Chromatography (VLC) with stationary phase using silica gel GF254 (Merck) and mobile phase using n-hexan : ethylacetate with various comparison gradient until 10 fractions were gained. Afterward, every fractions were analyzed using Thin-layer Chromatography (TLC) with standard pinostrobin (Sigma-Aldrich) as control. Then, fraction 4 (N-hexan : ethylacetate 35:15) was separated once more using Preparative Thin-layer Chromatography (Prep TLC) until pinostrobin was gained (67 mg). Pinostrobin obtained from the previos processes was analyzed using TLC Densitometry, so it could be confirmed that the isolates was Pinostrobin isolate.

HeLa cell cultures were obtained from Laboratorium Penelitian dan Pengujian Terpadu

(LPPT) Hayati Universitas Gadjah Mada.

The cultures were grown on RPMI media and completed with supplementation of penicillin-streptomycin (Penstrep® Sigma-Aldrich), Amphotericin B (Fungizone® Sigma-Aldrich), ceftriaxone 0,1% and *bovine serum* 10% (Sigma-Aldrich). Replacement of media was done everyday, while sub-culture was done when the cell compactness in the flask exceed 80%.

b. Intervention and MTT assay

HeLa cells were trypsinized with trypsin 0,25% and then washed with PBS 3 times, and later resuspended in fresh RPMI medium and moved into 96-well plate with density 5×10^3 cell/mL, with volume 200 μ L, incubated for 1 day until the cells attached and entered growth phase. Later on, the medium was changed with another medium that had been added with pinostrobin in the concentration of 5, 25, 50, 75, 100 and 250 μ g/mL (each concentration was triplicate), and then incubated for 24 hours. After that, the medium was changed again into fresh medium and incubated for 2 x 24 hours.

Afterward, MTT assay was done by: dispose

medium, change with 200 μ L fresh medium and added 50 μ L [3-(4,5 dimethyl thiazo-2-yl)-2,5 diphenyltetrazolium bromide] (MTT). Plate was wrapped in aluminum foil, incubated for 4-8 hours. Then, medium and MTT was disposed, the remaining crystal was dissolved in 200 μ L dimethylsulfoxide (DMSO). Added glycine buffer 25 μ L per preparations. Absorbance was read with ELISA reader in 570nm wavelength.

Data Analysis

The collected data was processed so that a greater percentage of cell viability at various pinostrobin concentration were achieved. Analysis was done using one-way ANOVA SPSS ver. 15, while inhibitory concentration (IC_{50}) of pinostrobin was determined by establishing a pinostrobin concentration curve against the percentage of viability.

RESULTS

In this study, MTT assay that was done on HeLa cultures with and without pinostrobin temu kunci or fingerroot, generates result as seen in Table 1.

Table 1. HeLa cell cultures viability

No.	Pinostrobin concentration	Viability (%) \pm SD	p (ANOVA)
1.	5 μ g/mL	92,58 \pm 9,84	$p = 0,00$
2.	25 μ g/mL	91,78 \pm 4,46	
3.	50 μ g/mL	80,09 \pm 4,51	
4.	75 μ g/mL	76,89 \pm 7,75	
5.	100 μ g/mL	67,85 \pm 11,31	
6.	250 μ g/mL	48,82 \pm 16,61	

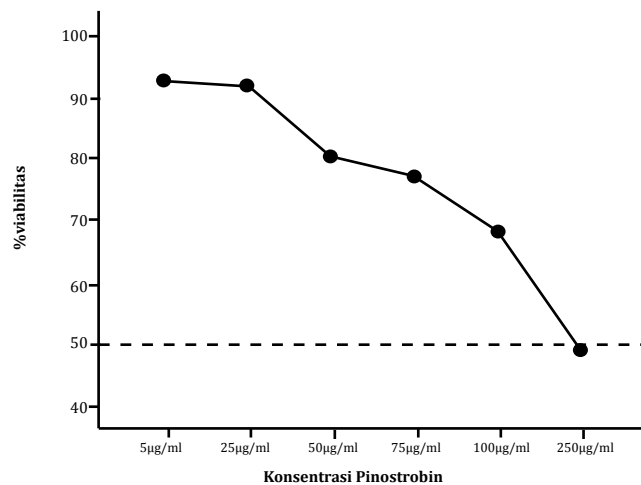


Figure 1. Graphic of HeLa cells viability against the addition of pinostrobin



Figure 2A. Normal HeLa cells



Figure 2B. HeLa cells with pinostrobin 25µg/mL



Figure 2C. HeLa cells with pinostrobin 50µg/mL

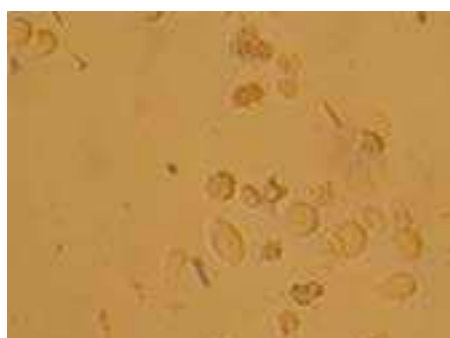


Figure 2D. Gambar2D. HeLa cells with pinostrobin 250µg/mL

Figure 2. HeLa cells with and without pinostrobin

On the graphic of HeLa cells viability against the addition of Pinostrobin (Figure1), it can be seen that pinostrobin concentration which inhibit 50% (IC_{50}) of cells viability is 250µg/mL.

DISCUSSION

In this study, it was found that the addition of pinostrobin on the culture of HeLa cells can significantly ($p = 0,00$) reduce the viability of cells. Based on posthoc test with Tukey HSD, significant difference can be seen in pinostrobin concentration 250µg/mL compare to other gradient of concentration, and between concentration 100µg/mL with concentration 5 and 25µg/mL.

This result implicate that Pinostrobin has low cytotoxic activity against HeLa cells. The cytotoxic activities are classified into four groups : High ($IC_{50} < 10 \mu\text{g/ml}$), active ($IC_{50} > 10\mu\text{g/ml} < 50\mu\text{g/ml}$), Moderate active ($50 < IC_{50} < 100\mu\text{g/ml}$), and inactive ($IC_{50} > 100\mu\text{g/ml}$).¹⁷ Hence, in this research it can be concluded that the cytotoxic activity of Pinostrobin is inactive.

The result of this research is dissimilar with

other researches who also studied the cytotoxic activity of Pinostrobin. Poerwono et al¹⁸ studied its effect on cancer cells SK-BR-3, MCF-7, and PC-3, obtained IC_{50} against 94,3 µM, 84,9 µM, and 86,7 µM. Sukardiman et al¹⁹ studied its effect on the culture of breast cancer cells, concluded that the anti-cancer effect of Pinostrobin is due to its inhibitory activity on topoisomerase I enzymes which contribute on DNA replication. Advanced research showed that Pinostrobin temu kunci or fingerroot also has apoptotic induction activity against human breast cancer cells T-47D through p53 and bax pathway, as well as inhibition on COX-2 expression in vitro.²⁰ The difference in findings between this research and previous researches might be due to a different response or sensitivity of HeLa cells against pinostrobin, but further research is needed to confirm this assumption.

CONCLUSION

Pinostrobin did not show any cytotoxic activity against the culture of HeLa cells.

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